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Local Anesthetics, Chlorpromazine and Propranolol Inhibit Stimulus-Activation of Phospholipase A_2 in Human Platelets

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SUMMARY

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Stimulation of platelets by various agents such as thrombin, collagen and A23187 leads to a rapid Ca^{2+} - dependent, phospholipase A_2-mediated mobilization of arachidonic acid from certain phospholipids and the biotransformation of this fatty acid to prostaglandin endoperoxides, thromboxanes A_2 and B_2, prostaglandins, hydroxyacids and malonyldialdehyde (MDA). Local anesthetics (dibucaine, tetracaine, benzocaine, and QX572), chlorpromazine and propranolol are very effective inhibitors of MDA formation induced by thrombin, collagen and A23187, but they do not block conversion of *exogenous* arachidonate to MDA. This result indicates that these drugs do not affect the enzyme prostaglandin synthetase responsible for the initial steps in arachidonate conversion to PG endoperoxides and thromboxanes. Utilizing platelets whose phospholipids were prelabeled with ^{14}C-arachidonate and then stimulated with thrombin or A23187 we have found that local anesthetics, chlorpromazine and propranolol block: (a) formation of ^{14}C-thromboxane B_2, ^{14}C-prostaglandins and ^{14}C-hydroxyacids (12-L-hydroxy-5,8,10,14-eicosatetraenoic acid and 12-L-hydroxy-5,8,10-heptadecatrienoic acid) from *endogenous* (phospholipid) ^{14}C-archidonate; and (b) block the fall in the ^{14}C-arachidonate content of platelet phospholipids, especially phosphatidyl choline. Local anesthetics, chlorpromazine and propranolol did not inhibit the conversion of ^{14}C-arachidonate to ^{14}C-12-L-hydroxy-5,8,10,14-eicosatetraenoic acid by a soluble fraction from platelets containing lipoxygenase activity. These results conclusively demonstrate that local anesthetics, chlorpromazine and propranolol inhibit the activation of PLA_2 by stimuli, which is the initial and rate-limiting step in arachidonate metabolism. To some extent the ability of local anesthetics, chlorpromazine and propranolol to inhibit platelet aggregation and the release reaction (secretion of ADP, serotonin, etc.) can undoubtedly be attributed to their effect on PLA_2 activation which prevents formation of the aggregating agents PG endoperoxides and thromboxane A_2. However, other actions of these drugs, notably their ability to block aggregation induced by thromboxane A_2 and by other aggregating agents, under conditions which are independent of PG endoperoxide and thromboxane A_2 production, indicates additional mechanisms of action which can prevent cellular adhesion and exocytosis. These other actions may be related to non-arachidonate mediated effects on Ca^{2+}-dependent phospholipases, antagonism of Ca^{2+}-dependent phenomena necessary for membrane adhesion and fusion, or interference with the process of intracellular Ca^{2+} release by stimuli.

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INTRODUCTION

Local anesthetics inhibit platelet aggregation and the secretion of contents of platelet storage granules elicited by ADP, thrombin, collagen and the Ca^{2+}-ionophore A23187 (1). Another response of platelets to stimulation involves the very rapid mobilization of arachidonic acid from phospholipid stores and the biotransformation of this fatty acid by two pathways (Fig. 1) to form several prostaglandins, hydroxyacids and thromboxanes (2). TXA_2[2] and the PG endoperoxides PGG_2 and PGH_2 are very labile intermediates which are themselves capable of evoking platelet aggregation and the release reaction (3). The concentration of free arachidonic acid in platelets is negligible. The rate-limiting step in the metabolic transformations of arachidonic acid appears to be the activation of the enzyme PLA_2 which releases arachidonate from certain phospholipids (4–7). PLA_2 requires Ca^{2+} for its activity (6) and the extent of activation of the arachidonate metabolic pathway is apparently in part a function of the degree to which free Ca^{2+} is mobilized within the cell by stimuli (7–9). Since local anesthetics may affect Ca^{2+} mobilization in platelets (1) and are also known to inhibit snake venom, seminal vesicle, pancreatic and mitochondrial phospholipases (10–12), we previously investigated their effects on the arachidonate pathway in human platelets. Dibucaine and tetracaine were found to inhibit the formation of MDA, a metabolite of arachidonic acid (Fig. 1), in platelets which had been stimulated by thrombin, collagen or A23187 (13). However, the formation of MDA from exogenously added arachidonate was not inhibited, indicating that the enzyme prostaglandin synthetase was not affected by local anesthetics. Dibucaine and tetracaine also inhibited the rapid burst in platelet O_2 consumption brought about by platelet-aggregating agents in the presence of antimycin A (13), which is due to oxidation of endogenous arachidonic acid. These experiments pointed toward a possible inhibition of PLA_2 as another pharmacological action of local anesthetics on platelets.

In this paper we report further experiments designed to conclusively determine if PLA_2 activation by platelet stimulating agents is inhibited by local anesthetics. In addition, a number of different chemical types of local anesthetics as well as chlorpromazine and propranolol (which possess significant local anesthetic activity) have been included in these studies.

[2] The abbreviations used are: TXA_2, thromboxane A_2; PG, prostaglandin; PLA_2, phospholipase A_2; MDA, malonyldialdehyde; BSA, bovine serum albumin.

METHODS AND MATERIALS

Metabolism of [^{14}C]-arachidonic acid-labelled washed platelet suspensions. To prepare labelled platelet suspensions, the procedure reported by Bills *et al.* (5) for incorporation of radioactive arachidonic acid into the endogenous platelet phospholipids was modified as follows: [^{14}C]-arachidonic acid (about 10^6 cpm) in hexane is evaporated under nitrogen and the residue is dissolved in an equal volume of ethanol followed by an equal volume of buffer. Washed platelets (about 20–40 mg protein) containing fatty acid-free BSA (4 mg/ml), or platelet concentrates in plasma, are added to the tube with the arachidonic acid and the platelets are incubated at 37° for 1 hr. After cooling to room temperature, sodium EDTA (pH 7.4) is added to a final concentration of 1 mM and the platelets are centrifuged at 1475 × *g* for 10 min at room temperature. The supernatant containing unincorporated arachidonic acid is removed, the platelet pellet resuspended in an equal volume of Tris/saline buffer (137 mM NaCl, 2.7 mM KCl, 0.2% w/v dextrose, 25 mM Tris-HCl, pH 7.5) and recentrifuged at 1475 × *g* for 10 min. The platelet pellet is finally resuspended in Tris/saline buffer to a concentration of 3 mg platelet protein/ml.

Extraction procedure. After the incubation of platelets with aggregating agents for a suitable time 0.5 ml 0.5M citric acid (pH 3.0) is added to the platelet suspensions. After the addition of about 0.5 ml water to facilitate phase separation, the mixture is extracted with 10 ml chloroform/methanol (1:1). The aqueous phase is reextracted with 5 ml chloroform and the organic phases combined. After several hours, any

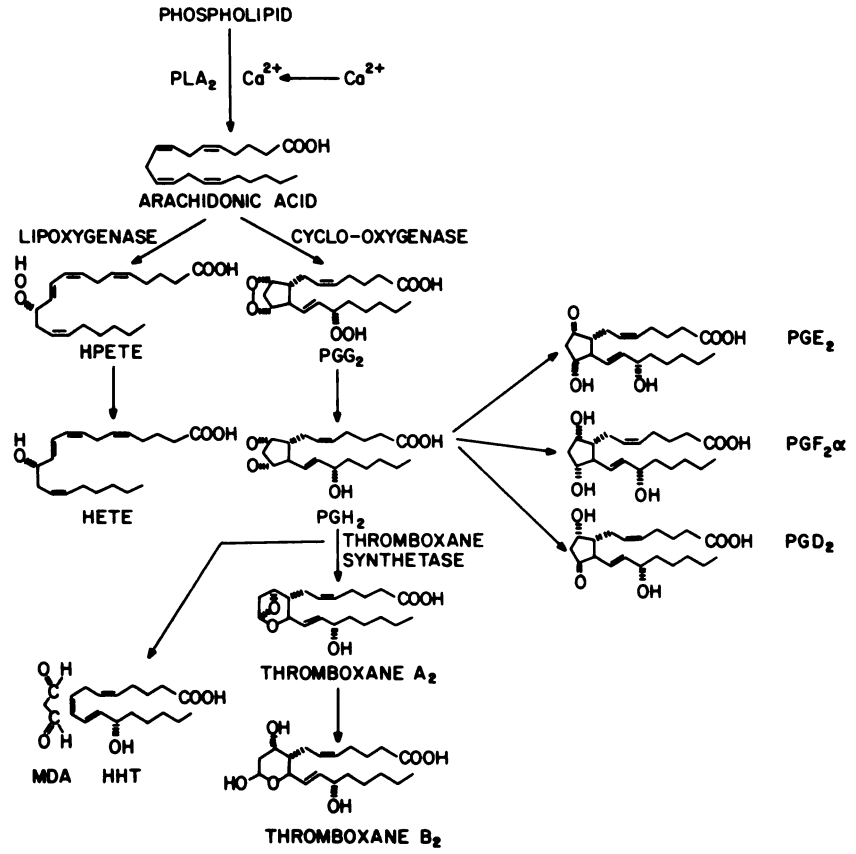

FIG. 1. *Metabolic pathways of arachidonic acid in platelets*

residual water is removed and the chloroform extract is evaporated under nitrogen. The residue is immediately redissolved in a small volume of chloroform.

Chromatographic procedure. The chloroform solution is applied to a column of silicic acid (0.5 g) preactivated at 100° for 1 hr and the following fractions are eluted: fraction 1 (PGs, hydroxy acids and arachidonate)—10 ml chloroform followed by 10 ml of 5% methanol in chloroform; fraction 2 (phospholipids)—6 ml methanol followed by 4 ml of 1% water in methanol. Both fractions are evaporated under nitrogen and the residues immediately redissolved in a small volume of chloroform/methanol (1:1). *Fraction 1* is applied to a silica gel G thin-layer plate previously activated at 110° for 1 hr and developed in ethyl acetate/acetic acid (99:1). Also included on parallel lanes on the plate are the following standards: PGA_2, PGD_2, PGE_2, $PGF_{2\alpha}$, TXB_2, ricinoleic acid and arachidonic acid. After initial development a second solvent line is drawn under the PGA_2 standard (previously located with iodine vapor) and the plate is redeveloped in the above solvent system. After the plate has been dried for 20 min, it is scanned with a Berthold TLC radioisotope scanner and then stained by spraying with 10% phosphomolybdic acid in ethanol and heating for 5 min at 110°. Zones are demarcated according to positions of the standards, scraped into vials and assayed for radioactivity in a liquid scintillation counter. *Fraction 2* is applied to a preactivated (110° for 1 hr) thin-layer plate coated with a 1:1 mixture of silica gel G and Supelcosil 42A. Also included on parallel lanes are the following standards: phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositide (PI), and phosphatidyl serine (PS). The plate is developed in chloroform/

methanol/concentrated ammonium hydroxide/water (65:35:2.5:2.5) and after drying for 20 min, it is scanned and then stained with iodine. Phosphatidic acid (PA) is not well separated from PS by this system. Zones are demarcated according to positions of the standards, scraped into vials, 5 ml of scintillation cocktail added (150 mg POPOP, 12 g PPO, 600 ml Triton X-100 in 2400 ml toluene) and assayed for radioactivity in a liquid scintillation counter.

Lipoxygenase activity. A soluble fraction containing lipoxygenase activity is prepared from sonicated platelets by centrifugation at 49,000 × *g* for 1 h to remove membranes (14). This enzyme preparation is incubated with [^{14}C]-arachidonic acid and the mixture extracted and assayed for metabolites of arachidonate as described above. Prostaglandin synthetase activity is not present in this fraction.

Chemicals, prostaglandins, thromboxane B_2 and phospholipids were obtained from Sigma Chemical Co. (St. Louis, Mo.) or were gifts from Dr. J. Pike (Upjohn Co.). [1-^{14}C]-Arachidonic acid was purchased from Amersham-Searle (spec. activity 56 mCi/mmole). Local anesthetics and chlorpromazine were purchased from ICN Pharmaceuticals Inc. and Sigma Chemical Co. QX 572, QX 314 and QX 222 were kindly supplied by Dr. Bertil Takman (Astra Pharmaceutical Products, Inc.). 5,8,11,14-Eicosatetrynoate was a gift from Dr. D. N. Whitman, Hoffman-LaRoche, Inc.

RESULTS

The local anesthetics dibucaine and tetracaine, as well as chlorpromazine and propranolol were each able to block MDA formation in thrombin-, collagen- and A23187-stimulated platelets. However, MDA formation from metabolism of *exogenous* arachidonate was unaffected by these agents indicating that prostaglandin synthetase activity was not inhibited. Dibucaine, chlorpromazine and propranolol produced detectable (10–20%) inhibition of maximal MDA formation induced by A23187 or thrombin at 50 μM, 10 μM and 33 μM, respectively. The K_I values (concentrations producing 50% inhibition of MDA formation) for these agents were: chlorpromazine 50 μM, propranolol 250 μM, dibucaine 125 μM.

Other local anesthetics which we found strongly inhibited (>90%) only MDA production from endogenous arachidonate, as a result of platelet activation by thrombin, included benzocaine (2 mM) and QX5732 (10 mM), a quarternary amine relative of lidocaine, with two benzene rings and greater lipid solubility. Lidocaine and QX 314 (N-ethyl lidocaine) inhibited thrombin-induced MDA formation 30–40% only at high concentrations, i.e., 15 mM. The effects of higher concentrations of lidocaine and QX 314 were not studied. These results indicate that tertiary and quarternary amine anesthetics, as well as benzocaine, which lacks an alkyl amine group, are all capable of inhibiting mobilization of platelet arachidonic acid. Potency may be related to lipid solubility of the drugs.

Effects of local anesthetics, chlorpromazine, and propranolol on metabolism of endogenous arachidonic acid. Washed platelets whose endogenous phospholipids had been previously labelled with ^{14}C-arachidonate were incubated with concentrations of thrombin or A23187 known to produce maximal MDA formation. Stimulation by these agents resulted in the increased formation of radioactive TXB_2, hydroxyacids (HETE:12-L-hydroxy-5,8,10,14-eicosatetraenoic acid, and HHT: 12-L-hydroxy-5,8,10-heptadecatrienoic acid) and prostaglandins. Prior exposure of platelets to dibucaine, tetracaine, chlorpromazine or propranolol at concentrations which blocked MDA formation, essentially abolished the increased production of ^{14}C-arachidonate metabolites resulting from platelet stimulation by thrombin or A23187 (Figs. 2,3). It is significant to note that the production of both the hydroxy fatty acids as well as that of TXB_2 and prostaglandins was affected (Fig. 3). HETE, a product of lipoxygenase activity, comprises the major fraction of the hydroxyacids formed from endogenous ^{14}C-arachidonate (15). However, the oxidation of ^{14}C-arachidonate to HETE by a soluble platelet lipoxygenase preparation was not affected by local anesthetics or chlorpromazine, but was com-

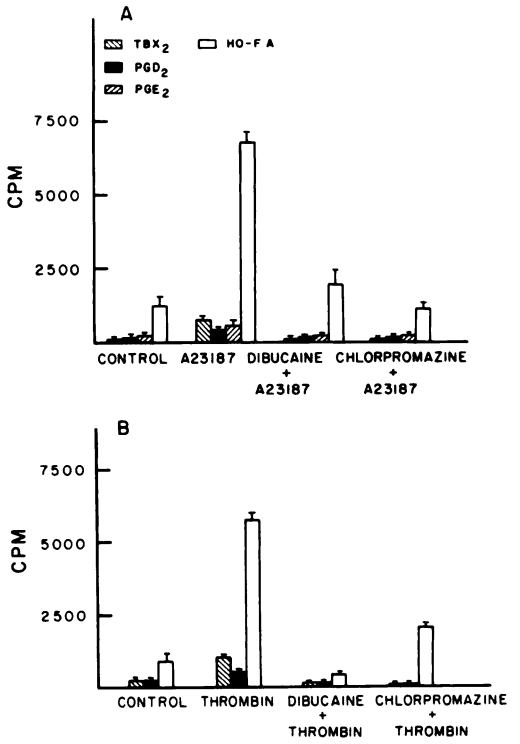

FIG. 2. *Formation of arachidonic acid metabolites from endogenous phospholipid [^{14}C]-arachidonic acid*

Washed platelets previously equilibrated with [^{14}C]-arachidonic acid to permit incorporation into phospholipids were exposed to (A) thrombin (5 U/ml) or (B) A23187 (5 μM). In typical experiments the effects of dibucaine (1 mM) or chlorpromazine (0.2 mM) added 2 min prior to platelet stimulation are shown. The data show the mean ± the range of duplicate experiments on a typical single batch of platelets.

pletely blocked by eicosatetraynoate, a known inhibitor of the lipoxygenase (Fig. 4).

Effects of local anesthetics, chlorpromazine and propranolol on thrombin- or A23187-induced mobilization of endogenous phospholipid ^{14}C-arachidonate. The distribution of ^{14}C-arachidonate incorporated into phospholipids *in vitro* is shown in Fig. 5. Phosphatidyl choline (PC) contained the greatest fraction of the ^{14}C-arachidonate incorporated into the platelet phospholipids. Phosphatidyl inositide and phosphatidyl ethanolamine were also significantly labelled, but phosphatidyl serine plus phosphatidic acid were poorly labelled, as previously shown (4, 5). Stimulation of platelets by thrombin or A23187 resulted in a substantial fall in ^{14}C-arachidonate content in the PC pool in all experiments. The magnitude of the decrease ranged from 30–50% in 8 experiments (Figs. 5, 6). In some experiments (Fig. 5A) a fall in ^{14}C-arachidonate content in PI and PE was also observed. The pool of ^{14}C-arachidonate in PS plus PA was essentially unaffected. Dibucaine, chlorpromazine and propranolol inhibited the thrombin- and A23187-induced release of ^{14}C-arachidonic acid from phospholipids (Figs. 5, 6). Their effects were most notable on the PC pool, but in those experiments where PI- and PE-^{14}C-arachidonate were also affected, the breakdown of those phospholipids was inhibited as well.

The amount of ^{14}C-arachidonic acid which disappeared from the phospholipid pool in thrombin- and A23187-treated platelets could be substantially accounted

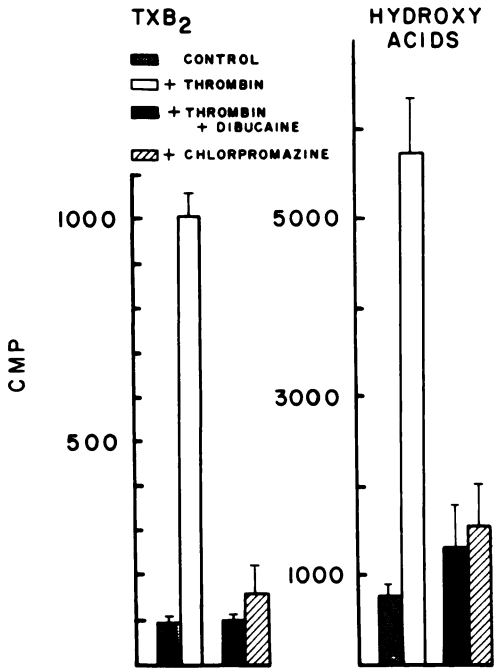

FIG. 3. *Effects of dibucaine (1.0 mM) and chlorpromazine (0.2 mM) on the formation of thromboxane B_2 and hydroxyacids (HETE + HHT) in thrombin-treated platelets, whose phospholipids were prelabelled with [^{14}C]-arachidonic acid*

The results are the means ± S.E.M. for 3–5 experiments on different platelet batches.

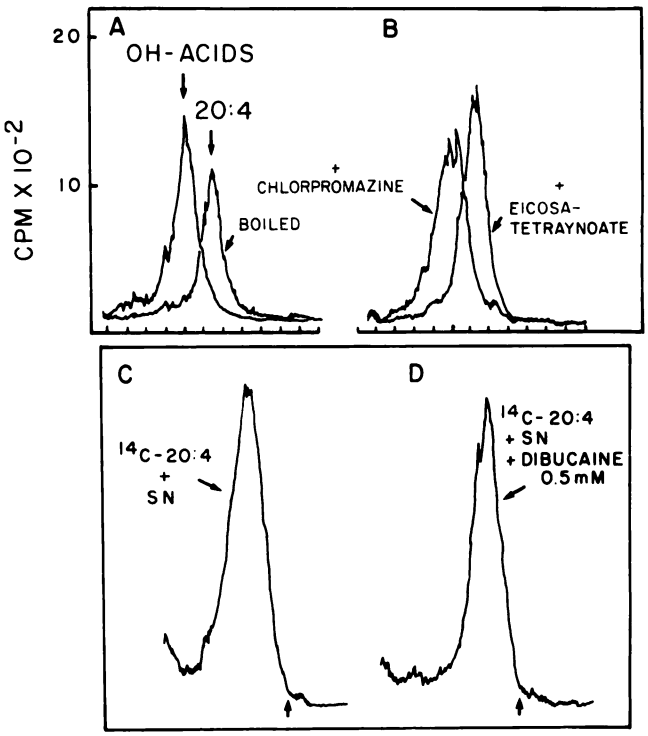

FIG. 4. *Effect of chlorpromazine, dibucaine and eicosatetraynoate on metabolism of [^{14}C]-arachidonic acid (3 μM) added to 1.0 ml soluble platelet fraction (3.2 mg protein/ml, Tris HCl 25 mM, pH 7.4) containing lipoxygenase activity*

Incubated for 5 min at 37°. Radioactivity scan of products on TLC plate in the region where the hydroxyacid 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE) and arachidonic acid (20:4) migrate: (A) show the presence of a peak for HETE produced from arachidonic acid in control soluble fraction and the peak found with the boiled fraction which contains no HETE. The latter peak corresponds exactly to that for [^{14}C]-arachidonic acid standard; (B) platelet soluble fraction pre-treated with 0.2 mM chlorpromazine or with 100 μM eicosatetraynoic acid (an inhibitor of lipoxygenase). The latter peak corresponds exactly to that for [^{14}C]-arachidonic acid indicating complete inhibition of lipoxygenase by eicosatetraynoate. The fraction treated with chlorpromazine contains only HETE with no detectable ^{14}C-arachidonate remaining. (C) Control: ^{14}C-arachidonate + soluble supernatant, (D) soluble fraction + ^{14}C-arachidonate in the presence of dibucaine (1.0 mM). In both cases (C and D) all the radioactivity is in the HETE fraction, no ^{14}C-arachidonate (position indicated by arrows) is detectable.

for in the amount of radioactivity present in the various metabolites (Table 1).

DISCUSSION

These experiments establish that certain local anesthetics, chlorpromazine and propranolol can inhibit the stimulus-induced breakdown of platelet phospholipids to form free arachidonic acid. As a result, the formation of metabolites of arachidonic acid, notably thromboxane B_2, prostaglandins, hydroxyacids, and malonyldialdehyde is prevented. Similarly, the formation of the transient aggregation-inducing metabolites, PG endoperoxides (PGG_2, PGH_2) and TXA_2 must also be inhibited. Since local anesthetics, chlorpromazine and propranolol do not affect the formation of MDA from *exogenous* arachidonic acid they must block arachidonate metabolism at a step prior to the prostaglandin synthetase (cyclooxygenase)-mediated reactions since MDA arises from the metabolism of PG endoperoxides (16) (Fig. 1). Furthermore, as stimulus-induced (i.e., thrombin, A23187) hydroxyacid (i.e., HETE + HHT) production is also blocked by these drugs, the site of action of the inhibitors must be

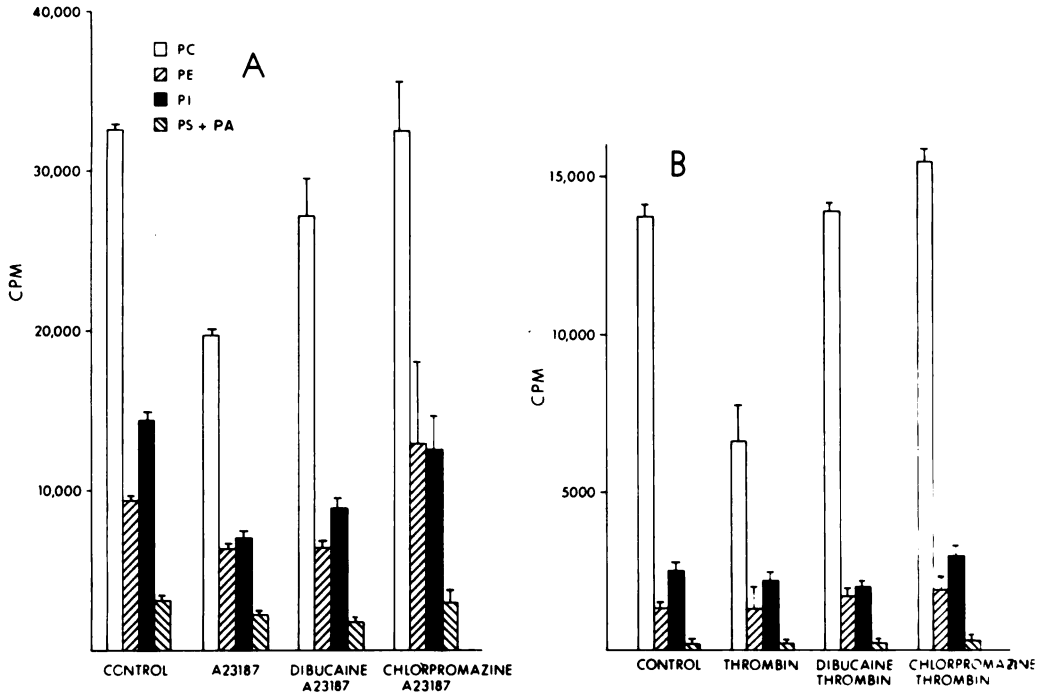

FIG. 5. *[^{14}C]-Arachidonic acid content in platelet phospholipids*

Effects of dibucaine (1.0 mM) and chlorpromazine (0.2 mM) on A23187 (5 μM) and thrombin (5 U/ml)-treated platelets prelabelled with ^{14}C-arachidonate. The data show the mean ± range for duplicate experiments on a typical single batch of platelets.

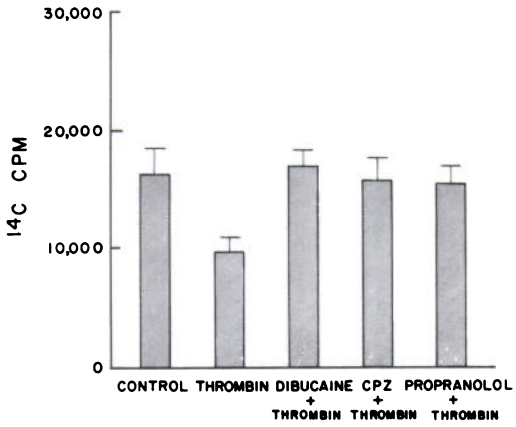

FIG. 6. *Effects of dibucaine (1.0 mM), chlorpromazine (0.2 mM) and propranolol (0.5 mM) on thrombin-induced fall in ^{14}C-arachidonate content of platelet phosphatidyl choline*

Data are mean ± S.E.M. for experiments on 3–5 different batches of platelets.

TABLE 1

Distribution of radioactivity in ^{14}C-arachidonic acid-labeled platelets

Washed platelet suspensions (1.0 ml, containing 3 mg platelet protein) previously pre-labelled with ^{14}C-arachidonic acid, were incubated at 37° with thrombin (5 U/ml) alone, or thrombin plus dibucaine (1.0 mM) or chlorpromazine (0.2 mM). After 5 min platelet suspensions were extracted and chromatographed as described in METHODS and the radioactivity in phospholipids and arachidonic acid metabolites was determined.

	cpm (total phospholipids)	cpm total arachidonic acid metabolites	Total cpm
Control	17,860	6,580	24,440
Thrombin	10,478	14,357	24,835
THR + dibucaine	17,853	5,040	22,893
THR + chlorpromazine	20,727	7,076	27,803

on PLA_2 activation because they do not affect lipoxygenase activity. Inhibitors of PG synthetase (i.e., aspirin, indomethacin) block HHT formation but this results in an equivalent increase in HETE formation *via* the lipoxygenase pathway.

Local anesthetics, chlorpromazine and propranolol have previously been shown to inhibit, as well as stimulate under some circumstances, phospholipase A_2 activity from snake venoms, pancreas or seminal vesicles (10–12, 17, 18). The effects of local anesthetics on these phospholipase activities were quite complex and were dependent upon time of incubation, concentration, structure of the anesthetic and Ca^{2+} concentration. In seminal vesicle homogenates tetracaine also inhibited overall endogenous production of prostaglandins, but not the conversion of added free arachidonic acid to prostaglandins (10), indicating that prostaglandin synthetase activity was unaffected. The inhibition of PG formation by tetracaine was overcome by increasing Ca^{2+} concentration. Inhibition of pancreatic and seminal plasma PLA_2 by local anesthetics also seemed to be competitive with respect to the Ca^{2+} required for enzymatic activity (17, 18) and the inhibition of rat mitochondrial PLA_2 by dibucaine has been attributed to a displacement of Ca^{2+} from the enzyme-Ca^{2+}-substrate complex (12).

PLA_2 activation in the platelet produced by thrombin, collagen and A23187 probably requires the mobilization of intracellular Ca^{2+} stores (7, 8, 13, 19). Local anesthetics appear to inhibit stimulus-induced mobilization of internal stores of platelet Ca^{2+} under some circumstances (1).[3] We therefore believe that local anesthetics, chlo promazine and propranolol are able to a tagonize the activation of PLA_2 by (1) dire inhibition of Ca^{2+}-dependent enzyme acti ity, or (2) preventing stimulus-induced m bilization of intracellular Ca^{2+} required f PLA_2 activation, or (3) a combination both effects. Measurement of drug effec on PLA_2 activity in platelet homogenat and isolated membranes could be useful t assess their potencies as direct enzyme i hibitors. However, this approach is not e tirely satisfactory for an understanding the situation in intact platelets for the fo lowing reasons: (a) the relevant actual fr Ca^{2+} concentrations in the cytoplasm stimulated platelets is not known, and (b the rate of arachidonate release from plat let membrane phospholipids in homog nates or isolated membrane fractions co taining high Ca^{2+} (10 mM) is much low (6) than the rapid burst of arachidonat liberation in stimulated intact platelets.

The ability of local anesthetics to preve PLA_2 activation could significantly contri ute to their inhibitory effects on platel aggregation and secretion *in vitro*. Aggr gation and the release reaction induced b *low* concentrations of ADP, thrombin, co lagen and epinephrine are susceptible t inhibition by indomethacin (25) indicati a significant role for PG endoperoxides an TXA_2 under these conditions. However, r sponses to *high* levels of platelet stimulan can occur despite blockade of prostaglandi synthetase activity by aspirin or indomet acin (25–28). Local anesthetics effectivel block platelet aggregation and secretion i duced by high concentrations of collage thrombin and A23187 which occur ind pendently of PG endoperoxide and TXA formation, and furthermore they block a gregation induced by addition of exogenou arachidonate or TXA_2 (13). Recent studi

[3] Additional experimental support for such a mechanism in intact platelets has been obtained from studies with N-ethylmaleimide (NEM) which causes a large production of MDA (20), prostaglandins (21) and PG endoperoxides (22) in intact platelets. The mercurial ethylmercurithiosalicylate (thimerosal) has a similar action. We have found (Feinstein and Fraser, unpublished results) that NEM and thimerosal produce a large release of intracellular Ca^{2+} measured by employing chlortetracycline as a fluorescent probe for membrane-bound Ca^{2+}. This release of Ca^{2+} was partially inhibited by chlorpromazine, dibucaine and propranolol at concentrations which inhibited NEM-induced MDA formation. The most straightforward interpretation of this result is that NEM and thimerosal act to release internally sequestered Ca^{2+} thereby activating PLA_2 to produce free arachidonic acid. In this situation local anesthetic inhibition of PLA_2 activation appears to be dependent in part at least on its interference with the intracellular release of Ca^{2+}. Although the source of Ca^{2+} release by NEM and thimerosal in platelets is unknown, Pfeiffer *et al.* (23) have recent described conditions under which NEM causes relea of Ca^{2+} from rat mitochondria. NEM can also inhib Ca^{2+} pumps, for example, as in muscle sarcoplasm reticulum (24). Local anesthetics were unable to i hibit maximal Ca^{2+} release by A23187 as measured b the chlortetracycline technique, yet strongly dimi ished MDA formation. In this case direct inhibition PLA_2 by local anesthetics is apparent.

have suggested that platelet aggregation may be mediated by non-prostaglandin phospholipase A_2-derived products (29) or by phospholipase activation, not involving a metabolite of arachidonate, but perhaps related to metabolism of phosphatidic acid (15). If these latter views are correct phospholipase inhibitors would be expected to block platelet activation by all types of stimuli, which is essentially what has been observed up to this time for local anesthetic drugs. On the other hand, the ability of local anesthetic drugs to inhibit platelet aggregation, secretion and the activation of PLA_2 may also involve their effects on dynamic physico-chemical cellular processes which are involved in membrane adhesion and fusion reactions (i.e., membrane fluidity and phase transitions, membrane Ca^{2+} binding, and cytoskeletal organization) as well as stimulus-evoked mobilization of internal calcium stores (e.g., see also ref. 30).

It is likely that the entire range of pharmacological effects of local anesthetic-like drugs on platelets are the result of a combination of *several* types of molecular interactions and not simply to be explained by a single mechanism. However, one of the significant effects of local anesthetics, chlorpromazine and propranolol is clearly to inhibit activation of PLA_2 by various platelet stimuli. A similar effect may be of some significance with respect to certain pharmacological actions of these drugs on other cells.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Mrs. Carol Fraser.

Note added in proof. Since the acceptance of this paper for publication two reports have been published demonstrating the presence in brain and platelets of Ca^{2+}-activated phospholipase C activity specific for diacylglycerophosphoinositides–diacyl GPI (Sun, G. Y. *et al., Lipids* **14**, 229–235, 1979; Rittenhouse-Simmons, S., *J. Clin. Invest.* **63**, 580–587, 1979). A product of this enzymatic activity is arachidonate-rich diglyceride which can then presumably be attacked by lipases to yield free arachidonic acid. In platelets the production of diglyceride from diacyl GPI preceded the loss of arachidonate from phosphatidyl choline. Since no phosphatide phosphodiesterase activity acting on PC, PS or PE has been found in platelets the release of arachidonate from PC is probably due to PLA_2 activity. The possibility however remains that local anesthetics, chlorpromazine and propranolol may also affect the specific diacyl GPI phosphodiesterase or a diglyceride lipase to prevent arachidonate mobilization from diacyl GPI.

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